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DECLARATION UNDER 37 C.F.R. § 1.131 OF SHUMING NIE

I, Shuming Nie, do hereby declare:

1.   I am one of the inventors of the subject matter disclosed and claimed in the above-identified patent application ("the present invention").

2.   The present invention was conceived of and reduced to practice prior to August 2, 2001.

3.   As merely an example of both the conception and reduction to practice of the present invention, Exhibit A is attached to this Declaration. Exhibit A is a true and accurate copy of a document prepared before August 2, 2001. Information irrelevant to the purposes of this Declaration has been redacted from Exhibit A as attached hereto.

4.   Exhibit A describes a concentration-gradient quantum dot according to the present invention. In particular, Exhibit A discloses the synthesis and characterization of ternary $CdSe_{1-x}Te_x$ semiconductor nanocrystals that can be tuned continuously over a wide spectral range

by changing the composition (i.e., the ratio of selenium to tellurium) during synthesis. Exhibit A discloses that the emission maximum can be tuned continuously from one extreme to the other by controlling the mole ratio of the injected stock solution without changing the actual size of the particle. In addition, Exhibit A discloses that the nanocrystals have a quantum yield of about 30-50% at room temperature.

5.   I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Date: _March 26, 2009_   _____
                                          Shuming Nie, Ph.D.

Exhibit
A

Composition-Tunable Photoluminescence Observed in Ternary Semiconductor Nanocrystals of Fixed Size

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Semiconductor nanocrystals are of considerable current interest due to their unique size-dependent properties that are not available from either discrete atoms or bulk solids.[1] Recent research has demonstrated the wide spectral ranges over which the photoluminescence (PL) of various nanocrystalline materials can be tuned simply by changing the particle size.[2,3] Other properties of interest are high quantum efficiencies, narrow and symmetric emission profiles, wide optical absorption bands, and large molar absorptivities. Furthermore, several groups have shown that these highly luminescent nanocrystals can be conjugated to biological molecules such as proteins and nucleic acids for ultrasensitive fluorescence detection and imaging.[4] However, current studies are primarily based on binary semiconductor materials where the emission wavelength is tuned by changing the particle size from about 1 nm to 8 nm. As a result, the largest nanocrystals are expected to have 512 times the volume and 64 times the surface area of the smallest particles. These large differences could cause major problems in bioconjugation and surface chemistry, as well as in the binding and reaction kinetics of nanocrystals to target molecules.

Here we report the synthesis and characterization of ternary $CdSe_{1-x}Te_x$ semiconductor nanocrystals that are highly luminescent at room temperature and could be used as constant-size biological labels. For such fixed-size nanocrystals, we show that the emission wavelength can be tuned continuously over a wide spectral range by changing the composition (i.e., the ratio of selenium to tellurium) during synthesis. A surprising finding is a nonlinear relationship between the emission wavelength (energy) and the nanocrystal composition. Observed as a "depression" in the emission energy, this nonlinear effect allows the preparation of ternary semiconductor nanocrystals with red and near-infrared emission wavelengths that extend well beyond the limits of the parent binary systems. In comparison with this work, previous reports of ternary semiconductor nanocrystal systems have not examined the composition effect in detail nor has such striking nonlinear behavior been observed.[5]

Ternary nanocrystals were synthesized by modifying a procedure reported previously.[5] Briefly, an appropriate quantity of a premixed trioctylphosphine stock solution containing selenium and tellurium in a given ratio was rapidly injected into a hot tri-n-octylphosphine oxide (TOPO) solution, containing a slight excess of dimethyl cadmium. In order to maintain a fixed size throughout the composition range (x=0 to x=1), we used the strategies of Peng, Alivisatos and coworkers[6] for size control and focusing. This was achieved by carefully controlling the concentrations of the reagents and the growth time.
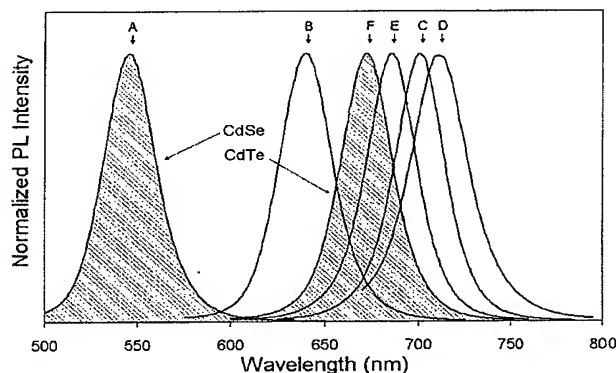

Figure 1. Photoluminescence spectra of $CdSe_{1-x}Te_x$ nanocrystals at a fixed size of 2.8 ± 0.3 nm (mean diameter) for compositions ranging from pure CdSe to pure CdTe. (A) x=0 , (B) x=0.20, (C) x=0.37, (D) x=0.62, (E) x=0.91, (F) x=1.0. The spectra were obtained from nanocrystals dispersed in chloroform using a fluorescence spectrometer (Spex) with an excitation wavelength of 475 nm.

Figure 1 shows the photoluminescence spectra for a series of $CdSe_{1-x}Te_x$ nanocrystals with a mean diameter of 2.8 ± 0.3 nm. At this particular size, the emission wavelength of pure CdSe nanocrystals is 547 nm, while that of pure CdTe nanocrystals is 672 nm. For the ternary nanocrystals, the emission wavelengths are considerably longer than those of the binary nanocrystals when the mole fraction of tellurium is between 0.35 and 0.90. With the longest emission wavelength at 712 nm and the shortest wavelength at 547 nm, the emission maximum can be tuned continuously from one extreme to the other by controlling the mole ratio of the injected stock solution without changing the actual size of the particle. To further examine how composition modulates the emission wavelength, we plot the PL energy as function of tellurium content (Figure 2). The curve reveals a strong nonlinear dependence and shows that the lowest energy gap (longest wavelength) occurs for nanocrystals whose composition corresponds to a mole fraction of approximately 0.62 in tellurium.

To confirm that the particle size is constant throughout the sample series, we obtained transmission electron microscopy (TEM) images of the nanocrystals (Figure 3). Statistical analysis of the TEM data indicates a constant particle size of 2.8 ± 0.3 nm (mean diameter). Powder X-ray diffraction patterns further reveal that the particles are highly crystalline and that the wurtzite structure is favored for all compositions under our synthetic conditions. In addition, we carried out elemental analysis by using inductively-coupled plasma mass spectrometry, which confirmed that the Se:Te mole ratio in the

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stock solution upon injection accurately reflects the actual composition of the nanocrystals within the experimental error associated with the preparation of the stock solutions. The composition values reported here correspond to the elemental analysis results.
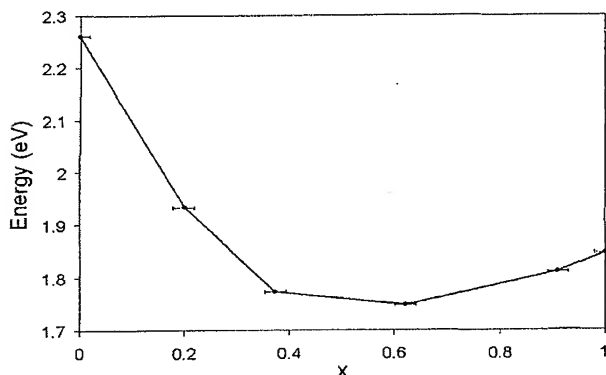

Figure 2. Plot of the band-gap energy as a function of composition for 2.8 ± 0.3 nm (mean diameter) $CdSe_{1-x}Te_x$ nanocrystals. The uncertainty in the composition determination is ±0.02 units as indicated by error bars.
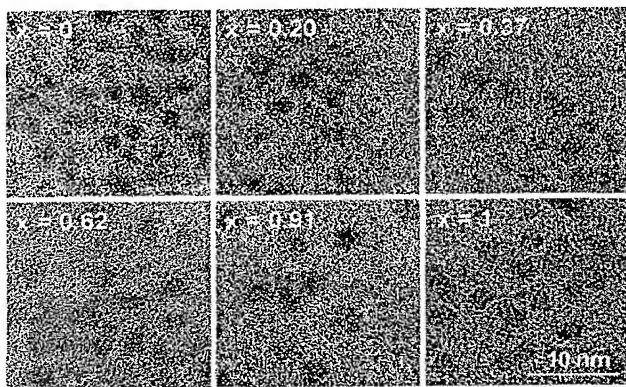

Figure 3. Transmission electron micrographs of $CdSe_{1-x}Te_x$ nanocrystals at various compositions. TEM images were acquired on a Philips CM200 microscope operated at an accelerating voltage of 120kV.

It is important to note the particle size variations and measurement errors are not large enough to account for the nonlinear relationship in Figure 2. Clearly, the observed variation in the band-gap is a composition effect. However, in our particular system there are two distinct sources which may contribute to this composition dependence. The first arises from the phenomenon that can be observed in the bulk material, where the band-gap energy changes as the alloy composition is varied.[7] The other can be traced to the quantum-confined nature of this nanocrystalline system. Recently, Poon et al. examined the composition-dependent optical properties of bulk $CdSe_{1-x}Te_x$.[8] They noted that this particular material exhibits a much greater nonlinear effect than other members of the mixed chalcogenide semiconductor family. In a model developed by Bernard and Zunger,[9] three

factors are believed to contribute to the observed nonlinear dependence: (i) a volume deformation which arises from the variation in the lattice constant of the changing alloy composition, (ii) a chemical-electronegativity effect, and (iii) internal relaxation of the anion-cation bond lengths in the alloy. This model has been used to explain the composition-dependent properties of bulk $CdSe_{1-x}Te_x$ with reasonable success.[8] These results indicate that the nonlinear, composition-dependent band-gap for the ternary nanocrystals is related to the behavior of the bulk system since these microscopic mechanisms are valid for both systems.

In addition to the characteristics inherited from the bulk material, the quantum-confined nature of these particles also contributes to the observed band-gap energy. The quantum-confined particle in our case is the optically generated exciton, whose size is dependent on the particular material in question. Therefore, even though we have maintained a constant nanocrystal size throughout our experiments, the size of the exciton, and subsequently the degree of confinement, could change as a function of the composition. A finite contribution is thus expected to arise from variations in the exciton confinement, but it is not possible to determine its magnitude based on the results presented here. To address this issue, we are carrying out systematic studies to examine this system over a wide range of particle sizes.

For biological applications, we note that the PL quantum yields of all samples appear to be composition-independent, ranging from about 5% to 10%.[10] After capping the ternary nanocrystals with CdS, the PL quantum efficiencies increase to about 30-50% at room temperature. The samples are highly monodisperse, as evidenced by their narrow emission profiles (full width at half maximum ~35 nm). By using the procedure reported by Chan and Nie,[4b] we have prepared water-soluble $CdSe_{1-x}Te_x$ nanocrystals. These crystals are highly stable and luminescent in biological buffer solution.

In conclusion, we have reported a simple strategy for preparing nanocrystals of a fixed-size that could serve as highly luminescent labels over a wide spectral range. An unexpected result is a nonlinear relationship between the emission wavelength and the nanocrystal composition. This nonlinear effect allows a broader range of spectral tuning than possible with the corresponding binary systems.[11] By keeping the particle size constant, the nanocrystals will have the same surface curvature, the same surface area, and the same diffusion rate and binding kinetics. We expect that luminescent ternary semiconductor nanocrystals will find applications in medical diagnostics, high-throughput screening, multi-parameter cell labeling, and multiplexed bead tagging.[12]

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(10) Quantum efficiencies were determined by comparing the integrated nanocrystal photoluminescence with that of Rhodamine 6G in ethanol.

(11) Recent work in our laboratory indicates that the tunable range can be extended to the near-infrared region by increasing the particle size. The longest wavelength we have observed is ~850 nm. Even broader spectral coverage could be obtained by using other ternary materials such as $CdS_{1-x}Se_x$, $CdS_{1-x}Te_x$, $ZnSe_{1-x}Te_x$, $Zn_{1-x}Cd_xTe$, $Cd_{1-x}Hg_xS$, etc.

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